

Claims

1. A method for providing an integrated genetic and physical map of a genome or part thereof, the method comprising the steps of:
- 5 (a) providing at least two individual genetic markers for the genome or part thereof, preferably in the form of a genetic map;
- (b) characterising the genetic markers by means of at least one AFLP fragment identified through AFLP fingerprinting;
- 10 (c) providing a library of clones comprising fragments of the genome or part thereof, preferably an artificial chromosome library such as a BAC or YAC;
- (d) generating a multitude of pools, each pool containing a multitude of individual clones from the library;
- (e) generating an AFLP fingerprint for each of the pools;
- 15 (f) identifying in the multitude of pools a pool in which an AFLP fragment identified in (b) is present in the fingerprint of the pool;
- (g) generating an AFLP fingerprint for each of the individual clones in the pool identified in (f), and identifying the clone containing the AFLP fragment identified in (b) in its AFLP fingerprint;
- 20 (h) generating a contig comprising the individual clone identified in step (g);
- (i) repeating steps (f-h) for at least a second AFLP fragment identified in (b) whereby the second or further AFLP fragments characterise a second or further genetic marker; and,
- 25 (j) linking at least two of the contigs obtained in (h) to thereby obtain an integrated physical and genetic map of the genome or part thereof, which comprises at least two genetic markers;
- wherein the forward and reverse AFLP primers used in step (b) and (e) comprise K respectively L selective nucleotides at the 3'-end of the primers, wherein the forward and reverse AFLP primers used in step (g) comprise M respectively N selective nucleotides at the 3'-end of the primers, wherein K, L, M, N are integers from 0 to 30 10, and wherein  $K+L \geq M+N$ .

2. A method for linking a genetic marker to a physical marker in a genome or part thereof, the method comprising the steps of:
- 5 (a) characterising the genetic marker by means of at least one AFLP fragment identified through AFLP fingerprinting;
  - (b) providing a library of clones comprising fragments of the genome or part thereof, preferably an artificial chromosome library such as a BAC or YAC;
  - 10 (c) generating a multitude of pools, each pool containing a multitude of individual clones from the library;
  - (d) generating an AFLP fingerprint for each of the pools;
  - (e) identifying in the multitude of pools a pool in which an AFLP fragment identified in (a) is present in the fingerprint of the pool;
  - 15 (f) generating an AFLP fingerprint for each of the individual clones in the pool identified in (e), and identifying the clone containing the AFLP fragment identified in (a) in its AFLP fingerprint;
  - (g) generating a contig comprising the individual clone identified in step (f), thereby linking the genetic marker to a physical marker;
- wherein the forward and reverse AFLP primers used in step (a) and (d) comprise K  
20 respectively L selective nucleotides at the 3'-end of the primer, wherein the forward and reverse AFLP primers used in step (f) comprise M respectively N selective nucleotides at the 3'-end of the primer, wherein K, L, M, N are integers from 0 to 10, and wherein  $K+L \geq M+N$ .
- 25 3. Method according to claim 2, wherein steps (a)-(g) are repeated for further genetic markers in the genome or part thereof and wherein the contigs obtained in (g) are aligned to thereby obtain an integrated physical and genetic map.
4. Method according claims 1-3, wherein  $(K+L) - (M+N)$  is at least 1,  
30 preferably at least 2, more preferably at least 3, most preferably at least 4.
5. Method according claims 1-4, wherein  $M+N$  is at least 0, preferably at least 1, more preferably at least 2, most preferably at least 3.

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6. Method according to claims 1-5, wherein each pool contains at most 0.6 genome equivalent of the total genome to be analysed, preferably 0.5 more preferably 0.4, most preferably 0.3.
- 5 7. Method according to claims 1-6, further comprising an additional pooling step .
8. Method according to claims 1-7, wherein the genetic markers are provided with a density of at least one genetic marker per 100 kb.
- 10 9. Method according to claims 1-8, wherein the contigs are aligned using a computer program suitable for aligning such as FPC
- 15 10. Method according to claims 1-9, wherein the artificial chromosome library contains at least 5 genome equivalents.
11. Use of AFLP primers for the integration of genetic and physical maps.
- 20 12. Use of AFLP in linking genetic and physical genome maps.
13. Use of AFLP primers in linking genetic and physical genome maps.
14. Use of a first and a second pair of AFLP primers in a method for linking genetic and physical genome maps wherein the first pair of AFLP primers comprise K respectively L selective nucleotides at the 3'-end of the primer, wherein the second pair of AFLP primers used comprise M respectively N selective nucleotides at the 3'-end of the primer, wherein K, L, M, N are integers from 0 to 10, and wherein  $K+L \geq M+N$ .
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